0022-202X/78/7403-0148$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 74:148–153, 1980

Vol. 74, No. 3
Printed in U.S.A.

Alteration of HeLa Cell Growth Equilibrium by Supernatants of Peripheral Blood Mononuclear Cells from Normal and Psoriatic Subjects

GERALD G. KRUEGER, M.D., AND WARREN W. JEDERBERG, M.S.

Division of Dermatology, Department of Medicine, University of Utah College of Medicine, Salt Lake City, Utah, U.S.A.

The possibility that products released by inflammatory cells may play a role in the induction/maintenance of psoriasis is suggested by the observation that psoriasis, a disease of excess epidermal proliferation, is linked to inflammatory events. As an assessment of this possibility, the effects of supernatants from antigen stimulated and unstimulated peripheral blood mononuclear cells from normal and psoriatic subjects on the proliferation of HeLa cell cultures are presented. Data demonstrate that supernatants contain factors which both inhibit and enhance cell proliferation, both of which are released in greater quantities from antigen stimulated peripheral blood mononuclear cells. Dilutional and pulsing experiments show that proliferation enhancing factors present in these supernatants have an apparent greater affinity for HeLa cells than does the inhibitory component. Relative to HeLa cell proliferation in fresh media, both antigen stimulated and control supernatants from peripheral blood mononuclear cells of subjects with psoriasis have significantly less inhibitory and more of the enhancing effect than similar supernatants from normal subjects. Individual, as well as pooled, supernatants from subjects with psoriasis demonstrate these differences. The kinetics of this response are the same, normal vs. psoriasis.

This "net effect" of supernatants from patients with psoriasis favoring proliferation is in harmony with the concept of inflammatory events playing a role in cell proliferation, and may be important in the induction/maintenance of psoriasis.

Using psoriasis as a model of benign proliferation disease, which is characteristically linked to inflammation, we are attempting to elucidate a mechanism where by mononuclear cells either enhance or suppress cell proliferation. Clinical observations have confirmed, on numerous occasions, that the abnormal epidermal proliferation of psoriasis is somehow related to inflammatory events, the Koebner reaction [1]. That mononuclear cells are potentially linked is suggested by the data which demonstrate that the early lesion of psoriasis is characterized by an infiltrate of mononuclear cells, occurring prior to the characteristic hyperplasia accompanying a typical lesion of psoriasis [2–4].

Although products produced by resting and stimulated peripheral blood mononuclear cells (PBMC) (recently termed lymphokines, from lymphocytes; and monokines, from monocytes) are usually thought of as modulators of the immune response, they effect cells other than leukocytes. PBMC products (still commonly referred to as lymphokines) directed against nonleukocytes include cytotoxic effects (lymphotoxin) [5,6], and inhibition of cell proliferation. The latter cytostatic lymphokines have been previously described under a variety of names, e.g. proliferation inhibition factor (PIF) [7,8], cloning inhibition factor (CIF) [9,10], interferon (IF) [11–13], inhibitor of DNA synthesis (IDS) [14–16], inhibitor of DNA polymerase (IDP) [17]. The clinical relevance of cytostatic lymphokine is essentially unexplored. Contrawise, monocytes produce factors (monokines) which enhance proliferation of cells other than leukocytes [18,19].

We have described other aspects of mononuclear cell function in psoriasis. Patients with psoriasis have a decreased ability to amplify delayed type intradermal skin tests to common antigens, and a decreased responsiveness to sensitization with dinitrochlorobenzene. PBMC from psoriatic subjects have a decreased blastogenic response in both the one-way mixed lymphocyte reaction, and to a wide range of dosages of mitogen [20]. When compared with monocytes from normal subjects, monocytes from patients with active and inactive psoriasis have an increased ability to migrate toward a variety of chemoattractants. In addition, monocytes from patients with psoriasis have an enhanced ability to spontaneously reduce nitroblue tetrazolium [21].

The foregoing observations of mononuclear cell function in psoriasis caused us to make a more direct assessment of a potential aberration in soluble products of mononuclear cells from psoriatic subjects on cell proliferation. No well-defined assay exists for quantitating the effect of either inhibitory or potentiating agents on epidermal cell proliferation *in vitro*. HeLa cells have been used by several investigators to determine the effect of soluble products of mononuclear cells ("lymphokines") on the growth of these cells *in vitro* [7,8,22].

This paper presents the results of a methodology which semiquantitatively assessed the amount of cytostatic activity in antigen and nonantigen stimulated mononuclear cell supernatants from normal and psoriatic subjects. These supernatants were added to HeLa cell cultures and tritiated thymidine uptake was utilized to determine the effect of these supernatants on HeLa cell proliferation. Evidence is presented which demonstrates that supernatants of cultured PBMC enhanced proliferation of HeLa cells, and that PBMC from psoriatic subjects produced more of said factor than did those from normal subjects.

METHODS

Experimental Subjects

Informed consent, obtained in accordance with the principles of the Declaration of Helsinki, was obtained from 13 psoriatic and 13 normal subjects. They had similar mean ages, age ranges, and sexual distribution. All psoriatic subjects had their disease for at least 5 yr, and had not received topical or systemic therapy for at least 30 days prior to skin testing, or PBMC donation. Psoriatic subjects were chosen from a group of volunteers meeting the aforementioned requirements. They had varying degrees of clinically active disease; the mean area of skin surface involvement being 20%. Five subjects had less than 10% of their body surfaces involved, and 6 had greater than 30% involvement. None

Manuscript received June 5, 1979; accepted for publication September 21, 1979.

This work was supported by grants from the National Institutes of Health, #2-P01-AM15740-04, 1-R01-AM21405-01.

Reprint requests to: Dr. Gerald G. Krueger, Division of Dermatology, University of Utah, Salt Lake City Utah 84132.

Abbreviations:
CPM: counts per minute
Derm O: Dermatophyton O
HBSS: Hank's balanced salt solution
PBMC: peripheral blood mononuclear cells
PIF: proliferation inhibition factor
PPD: tuberculin purified protein
SKSD: streptokinase, streptodornase

of the patients were undergoing an exacerbation of disease, and none had arthritis.

Lymphokine/Production

The technique for production and detection of cytostatic lymphokine was adopted from the method described by Green et al [7]. Our usage of the term "lymphokine" implies the original usage of this term, i.e., products released by PBMC, monocytes and lymphocytes. An operational definition of PIF has been given to lymphokine preparations with cytostatic activity *in vitro*. Chemical purity is not implied, nor is the presence of other lymphokine activity excluded by this designation.

Four weeks after skin testing, as previously described [20], 500 ml of blood (heparin 10 units/ml) (Hepathrom, Fellows Medical Mfg. Inc., Anaheim CA) were collected from each of the subjects. This was diluted 1:2 in Hank's Balanced Salt Solution (HBSS), placed on Ficoll-Paque (Pharmacia, Piscataway NJ) and centrifuged at 400 $\times g$ for 20 min [23]. Mononuclear cells isolated from the interface were suspended in RPMI-1640 (Gibco, Grand Island NY), supplemented with gentamycin, preservative free, 50 μg/ml (Schering Diagnostic Labs, Kenilworth NJ), and 15% AB+ sera (UUMC Blood Bank, SLC UT). Differential, viability and cell numbers were determined, and a final suspension of 1×10^6 PBMC/ml prepared. As previously noted, no differences were noted in the percentage of monocytes or lymphocytes isolated from normal and psoriatic subjects, as judged by microscopic analysis of cells stained with Wright's stain [20,21]. The PBMC suspension from each individual was divided into 3 aliquots, labeled A, B, and C. The antigen that caused the greatest induration on skin testing for that patient was added to Aliquot A. The antigens used were: Streptokinase 38 units/ml, streptodornase 12 units/ml (SKSD) (Lederle Laboratories, Pearl River NY), tuberculin purified protein (PPD) 10 μg/ml, or dermatophyton O (Derm-O), a filtrate from *Candida albicans*, 0.01 ml of a 1:10 dilution/ml (Hollister-Stier, Spokane WA). These antigen preparations were dialyzed extensively to remove preservatives, sterilized by filtration, and were the same as those used for skin tests. All aliquots were cultured in 30 ml volumes in tissue culture flasks in a humidified incubator, 5% CO_2, 37° for 96 hr. Aliquot B was treated in the same fashion as Aliquot A; however, antigen was not added until after supernatants were separated from the cells at 96 hr. Aliquot C was treated in a manner similar to Aliquot B, only no antigen was added at 96 hr. Supernatants from these incubations were sterilized by filtration and one-half of each aliquot was dialyzed twice against 20 vol of HBSS and once against 20 vol of RPMI-1640 penicillin 100 units, streptomycin 100 μg/ml (RPMI-PS) (Gibco, Grand Island NY). Dialysis did not effect the protein concentration, using the Lowry method for quantitation of protein content. Aliquots of individual PBMC supernatants, dialyzed and nondialyzed, were pooled and sterilized by filtration. All samples were stored at −70°C.

Semiquantitative PIF Microassay

Previously described techniques for assaying PIF activity require large volumes of supernatants of stimulated PBMC [7]. Because of volume restrictions we developed a microtechnique to assay PIF activity. HeLa (Ohio) cells (Microbiological Associates, Bethesda MD) were grown in RPMI-1640-PS with 10% fetal calf sera (Gibco, Grand Island NY) and used as targets after it could be demonstrated that confluence of cell growth could be achieved within 24 hr after a 1:2 dilutional passage. Assays were started within 2 hr of passage. HeLa cells, $5 \times 10^4 \pm 5\%$, suspended in 0.1 ml RPMI-1640-PS enriched with fetal calf sera (15%) were added to each well of a flat-bottom microtiter plate (Flow Laboratories, Englewood CA). Following the addition of HeLa cell suspensions, the plates were placed in a humidified incubator, 37°, 5% CO_2. After 4 hr of culture, 0.1 ml of a freshly thawed supernatant of a PBMC culture was added to each well. Plates were returned to the incubator; at 24 hr, 0.25 μCi of 3H thymidine specific activity 6.7 Ci/mM (New England Nuclear, Boston MA) were added to each well for the final 24 hr. Cells from each well of the microtiter plate were harvested with a multiple automated sample harvester unit (MASH II, Otto Hiller, Madison WS). Following the initial harvest, 50 μl of trypsin, 0.25% (Gibco, Grand Island NY) were added to each of the wells for 20 min, and wells reharvested with the MASH unit. Both filters from the harvest of the individual wells were placed in the same vial, scintillation fluid added (NEN-950 A, New England Nuclear, Boston MA) and radioactivity determined with a Beckman 8100 scintillation counting system. Microscopic examination of harvested wells revealed that essentially none of the cells adhered to the microtiter plates following the second harvest. Day to day variability of baseline counts occurred, as much as 50%. However, the amount of stimulation or suppression seen with a particular test supernatant was relatively constant. Because of this variability, data are presented as the percentage of the amount of 3H thymidine incorporated into HeLa cells grown in the presence of the fresh media controls for that experiment, using the following formula:

$$\text{percentage} = \frac{\text{CPM of 3H thymidine uptake by HeLa cells in test supernatants}}{\text{CPM of 3H thymidine uptake by HeLa cells in fresh media}} \times 100$$

This type of index permits the expression of potential stimulation (greater than 100%), as well as inhibition (less than 100%), of HeLa cell proliferation by the PBMC supernatants.

RESULTS

The Effect of Undialyzed Supernatants on 3H Thymidine Uptake by HeLa Cells

Conditioned or spent media can stimulate proliferation cells in culture. Therefore, the effect of nondialyzed, pooled supernatants ("conditioned media") from antigen-stimulated (active) and nonantigen stimulated (control) PBMC of normal and psoriatic subjects on the replication of HeLa cells maintained in tissue culture, was assessed by 3H thymidine incorporation. The data of Table I generally show that active supernatants of antigen-stimulated PBMC from both normal and psoriatic subjects, have more cytostatic activity than the corresponding control supernatants. At the lower dilutions, both active and control supernatants from psoriatic subjects are significantly

TABLE I. *Effect of undialyzed pooled lymphokine[a], active and control[b], normal vs. psoriasis, on 3H thymidine uptake by HeLa cell cultures: Percent of media[c]*

Lymphokine source	Dilution of test lymphokine[d]			
	1:1	1:4	1:16	1:64
Normal active	25 ± 8[e]	41 ± 8	56 ± 10	64 ± 10
Normal control	40 ± 6	51 ± 10	73 ± 13	72 ± 11
Psoriasis active	54 ± 10	73 ± 16	82 ± 26	103 ± 20
Psoriasis control	85 ± 18	105 ± 22	109 ± 21	107 ± 21

[a] Like samples pooled from 13 normal and 13 psoriatic subjects.

[b] Active = supernatants harvested at 96 hr from antigen-stimulated PBMC. Control = supernatants harvested from PBMC without Ag at 96 hr and antigen reconstituted.

[c] Data expressed as a percent of uptake of HeLa cells in media alone ± SEM. Each value is mean of supernatants on 5 HeLa cell cultures—each done in triplicate.

[d] Supernatants diluted with fresh media RPMI-1640 with penicillin and streptomycin.

[e] Significance (paired *t*-test, 2-tail).

	1:1	1:4	1:16	1:64
Normal active vs. psoriasis active	<0.02	<0.05	NS	<0.05
Normal control vs. psoriasis control	<0.05	<0.05	NS	NS
Normal active vs. normal control	<0.05	NS	<0.02	<0.1 < 0.05
Psoriasis active vs. psoriasis control	<0.1 < 0.05	<0.01	NS	<0.1 < 0.05

less inhibitory than similar supernatants from normal subjects. When supernatants are diluted with fresh media to 1:64 and more, the inhibitory activity is lost. There is no difference in activity between the PBMC supernatants that have been reconstituted with antigen, and those not reconstituted (see Methods, Aliquots B and C). The addition of antigen to HeLa cell cultures does not effect 3H thymidine incorporation; therefore, the nonantigen reconstituted lymphokine (Aliquot C) is not included in data analysis.

Effect of Dialyzed Supernatants on 3H Thymidine Uptake by HeLa Cell Cultures

To further assess the potential role of these observations being secondary to spent media effects, pooled supernatants were dialyzed against fresh media and assayed on 7 separate occasions in triplicate (see Table II). The normal control, psoriasis active, and psoriasis control, supernatants do not have as much inhibitory activity as the undialyzed component. However, the uptake of 3H thymidine with normal active supernatants is similar to that seen with the undialyzed counterpart. At the 1:1 dilution, the active supernatants from psoriatic subjects are much less inhibitory (85% of the uptake seen in cells grown in media) than the active supernatants from normal subjects (29%). As the supernatants are diluted, enhancement of 3H thymidine incorporation occurs. Beyond 1:64, 3H thymidine incorporation begins to return to baseline, i.e., that seen with HeLa cells grown in media. At these higher dilutions, the significant dichotomous differences, normal vs. psoriasis, are no longer present. Baseline is reached at dilutions of 1:500 or more. When data are analyzed, not as a percentage of activity in media alone, but as an index of activity of control supernatants (CPM active supernatants/CPM control supernatants), separately for normal and psoriatic subjects, psoriatic subjects are noted to produce approximately 30% less PIF activity than control subjects at the 1:1 dilution ($p < 0.02$, paired t-test, 2-tail). As the supernatants are diluted, these indices become similar, normal vs. psoriasis.

Analysis of Individual Supernatants and 3H Thymidine Uptake by HeLa Cell Cultures

To determine whether the pooled data were representative of the individual supernatants, active and control dialyzed supernatants from each individual subject were tested on 2 occasions in triplicate. Some of the individual supernatants displayed their most inhibitory or least inhibitory effect as concentrations other than those expected, i.e. 1:1 or 1:64. Therefore, data are presented as mean values of the concentrations that are most inhibitory and least inhibitory, Table III. These data demonstrate significant differences between normal and psoriasis, and confirm the data of the experiments where pooled supernatants were used.

Comparison of the Effect of Supernatants on Cell Proliferation

To determine whether 3H thymidine uptake accurately reflects the effect of the supernatants on cell proliferation in these experiments, assessment of the actual number of cells was carried out via microscopic analysis (see Table IV). When compared with media alone, the undiluted, pooled, dialyzed supernatants from normal subjects were very effective in inhibiting the proliferation of cells at 48 hr, $p < 0.01$. On the other hand, both supernatant preparations from psoriatic subjects, as well as the control supernatants from normal subjects, showed enhancement.

Kinetics of Inhibition vs. Enhancement of HeLa Cell Proliferation by Supernatants

The following experiment was undertaken to determine whether there were differences in the kinetics of inhibition or enhancement of proliferation by these supernatants. The effect

TABLE II. *Effect of dialyzed pooled lymphokine[a], active and control[b], normal vs. psoriasis, on 3H thymidine uptake by HeLa cell cultures, percent of media[c]*

Test lymphokine	Dilution of test lymphokine[d]			
	1:1	1:4	1:16	1:64
Normal active	29 ± 5[e]	69 ± 10	101 ± 17	120 ± 20
Normal control	86 ± 23	105 ± 20	128 ± 23	138 ± 25
Psoriasis active	85 ± 17	91 ± 16	120 ± 23	132 ± 24
Psoriasis control	124 ± 24	144 ± 27	157 ± 26	160 ± 30

[a] See Table I—Supernatants dialyzed × 3 - See Methods.
[b] See Table I.
[c] See Table I: n = 7 in this experimental series.
[d] See Table I.
[e] Significance (paired t-test, 2-tail).

	1:1	1:4	1:16	1:64	< 1:64
Normal active vs. psoriasis active	<0.02	<0.05	<0.02	NS	NS
Normal control vs. psoriasis control	NS	<0.02	NS	<0.1 < 0.05	NS
Normal active vs. normal control	<0.05	<0.02	<0.01	<0.02	NS
Psoriasis active vs. psoriasis control	<0.02	<0.05	<0.05	NS	NS

TABLE III. *Effect of individual aliquots of lymphokine[a], active and control[b] normal vs. psoriasis, on 3H thymidine uptake by HeLa cell cultures[c]*

Mean values of individual supernatants at most inhibitory and least inhibitory concentration			
Most Inhibition	% of Media ± SEM		
Psoriasis active	74 ± 6	55 ± 4[d]	Normal active
Psoriasis control	94 ± 3	65 ± 5[e]	Normal control
Least inhibition			
Psoriasis active	105 ± 4	87 ± 4[d]	Normal active
Psoriasis control	115 ± 4	90 ± 5[f]	Normal control

[a] Individual aliquots dialyzed (12 psoriasis, 13 normal) tested without pooling.
[b] See Tables I and II.
[c] Data expressed as the mean % uptake of 3H thymidine c.f. media alone of 2 different triplicate assays.
[d] $p < 0.05$ (Student t-test, 2-tail).
[e] $p < 0.001$ (Student t-test, 2-tail).
[f] $p < 0.005$ (Student t-test, 2-tail).

of pulsing with the various pooled dialyzed supernatants for 24 hr, followed by a pulse with media for 24 hr, is illustrated in the Figure, *a*. This is compared with an experimental procedure where media was added initially and replaced by their respective pooled supernatant at 24 hr (Figure *c*). The pattern of normal active and normal control supernatants having more inhibitory activity than the counterparts from patients with psoriasis is again apparent. Both the inhibitory and proliferative phenomena are present when HeLa cells were initially pulsed with supernatants and then replaced with media (Figure, *a*). However, when media was initially added, followed by supernatants at 24 hr, neither the inhibitory nor the proliferative effects are apparent (Figure, *c*). Data in Figure 1 are expressed as CPM's to illustrate the variability within an assay, as well as to illustrate the effect of pulsing with supernatant.

The kinetics of the reaction and the ability of HeLa cells to bind proliferative factor(s), as well as inhibitory factor(s), appear to be the same, normal vs. psoriasis (Figure). To ascertain whether PBMC supernatants might have their effect by not permitting HeLa cells to adhere, supernatants were added prior to (no 4-hr pre-incubation of HeLa cells in media) the addition of HeLa cells (data not presented). One or 4 hr after the addition of supernatants, the plates were centrifuged and supernatants removed, and replaced with media, new supernatants, or the supernatants were left in place. Centrifugation did not effect 3H thymidine incorporation, i.e., cells allowed to settle in the wells, or forced to settle with centrifugation, had very similar values, relative to their respective supernatants. These additional data show that either a 1 or 4 hr pulse, without the 4 hr presettling of the HeLa cells had the same effect as the 24-hr pulse. This, relative to both the inhibition and enhancement of cell proliferation for supernatants of normal and psoriatic subjects (Figure).

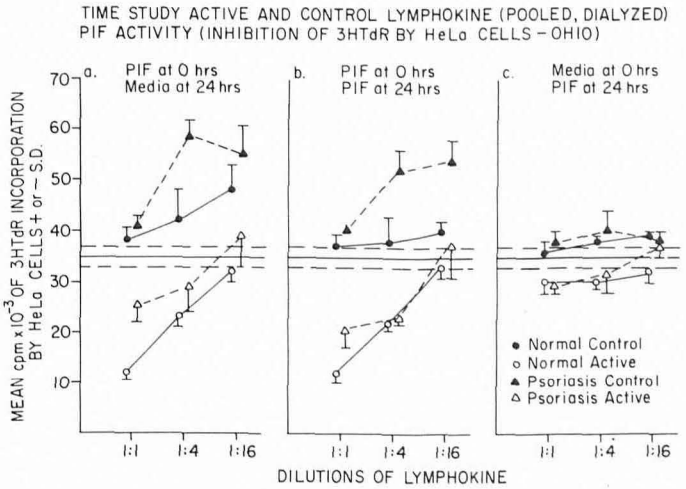
TABLE IV. *Comparison of the effect of dialyzed pooled lymphokine[a], active and control[b], normal vs. psoriasis, on proliferation of HeLa cells in culture*

	# of cells ($\times 10^5$)[c]	% of media[d]
Media alone at time 0	0.5 ± 0.05	--
Media alone at 48 hr	2.34 ± 0.05	--
Normal active lymphokine	1.36 ± 0.10	57[e]
Normal control lymphokine	3.01 ± 0.12	130[e]
Psoriasis active lymphokine	2.57 ± 0.16	109[f]
Psoriasis control lymphokine	3.06 ± 0.07	130[e]

[a] See Table I.
[b] See Table I.
[c] Actual # of cells added per well of 24 well costar plate—mean of duplicate experiments ± SEM.
[d] $\frac{\#\text{ of HeLa cells grown in media alone}}{\#\text{ of HeLa Cella grown in presence of lymphokine}} \times 100$.
[e] Significance $p < 0.05$ c.f. media (by Chi-square analysis).
[f] Significnce $p < 0.05$ c.f. normal active (by Chi-square analysis).

The Effect of Washing, Following a 4-hr Pulse with Supernatants

In an effort to differentiate the inhibition and enhancement seen in this experiment, cultures were pulsed for 4 hr with the various pooled dialyzed supernatants or fresh media and washed 2 times by centrifugation and resuspension in fresh media. Results are compared with cultures treated in a similar manner, but not washed (Table V). A 4-hr pulse, Panel A, Table V, demonstrates the expected effect. Panel B suggests that washing times 2, and then replacing with fresh superna-



Pulse study: *Panel a*—Lymphokine (*PIF*) added initially, withdrawn at 24 hr and replaced with media. *Panel b*—Lymphokine (*PIF*) added initially, withdrawn and replaced with lymphokine (*PIF*) at 24 hr. *Panel c*, Media added initially, withdrawn and replaced with lymphokine (*PIF*) at 24 hr. Values (cpm $\times 10^{-3}$) represent mean of an experiment done in triplicate ± SD.

TABLE V. *Effect of washing HeLa cells[a] after 4-hr pulse[b] on 3H thymidine incorporation, comparing dialyzed lymphokine, active and control, normal vs. psoriasis[c], at 1:1 dilution, percent of media[d]*

A. Lymphokine (PIF) added at time 0, centrifuged at 4 hr—supernatants not removed		
Lymphokine Source		
Normal active	38 ± 4	
Normal control	53 ± 8	
Psoriasis active	55 ± 9	
Psoriasis control	115 ± 11	
B. Lymphokine added at time O, removed at 4 hr, replaced with fresh original supernatants		
Lymphokine Source	No Wash	Wash ×2
Normal active	47 ± 16	75 ± 11
Normal control	44 ± 18	69 ± 5
Psoriasis active	52 ± 10	105 ± 3
Psoriasis control	102 ± 7	103 ± 4
C. Lymphokine added at time 0, removed at 4 hr, replaced with fresh media		
Lymphokine source	No wash	Wash ×2
Normal active	58 ± 8	143 ± 12
Normal control	83 ± 12	86 ± 16
Psoriasis active	84 ± 3	173 ± 3
Psoriasis control	159 ± 15	91 ± 6

[a] See Methods—HeLa cells added to chambers 4 hr prior to addition of supernatants.
[b] 4 hr after supernatants added, microtiter plates centrifuged and: (A) supernatants not removed, (B) supernatants removed and replaced with same fluids as added initially, (C) supernatants removed and replaced with media; In experiments B and C, 1 set washed ×2, 1 set not washed.
[c] See Tables I and II.
[d] See Table IV, values are mean of duplicate experiments performed in triplicate. Mean CPM in media alone in No Wash series = 25,800; and in Wash series, i.e., with fresh media each time = 26,900; in centrifuge without wash, centrifuge ×2 = 26,100; centrifuge ×3—28,400.

tants identical to those of the initial 4-hr pulse provides an advantage to factors which enhance the proliferation. Washing the cells times 2 to remove the supernatant effect again demonstrates that the enhancing effect is preferential, Panel C. Differences between the wash and no-wash experiments are significant ($p < 0.01$, paired t-test, 2-tail, Panel C, Table V). These data are in harmony with the concept that HeLa cells have preference for the factor(s) in supernatants which enhances the uptake of 3H thymidine. Further, more of the factor(s) which enhances proliferation is made when PBMC are antigen-stimulated than when not stimulated. This is demonstrated in the wash column of Panel C, Table V, where antigen-stimulated lymphokine shows more enhancement than nonantigen stimulated controls for both normal and psoriasis (143% vs. 86%, and 173% vs. 91%, respectively).

DISCUSSION

Clinicians feel that epidermal hyperplasia routinely follows inflammatory injury to the skin; however, experimental evidence for this observation, other than routine histology, is lacking [24,25]. Mononuclear cells, particularly macrophages, and their soluble products, appear to be vital in the proliferation which accompanies wound healing [18]. That these factors also cause normal epidermal hyperplasia, or the induction/maintenance of lesions of psoriasis, is plausible, but also, as yet, without experimental evidence. Data in this paper correlate with what would be expected, mononuclear cells from patients with a large part of their body surfaces involved in a disease of excess cellular proliferation produce less of the factor(s) which inhibits the proliferation of HeLa cell cultures (Tables II and V).

The concept of stimulated mononuclear cells producing factors which can both enhance and suppress cell proliferation for the immune system has gained ready acceptance [26]. However, the idea that such products inhibit cell proliferation, other than the immune system, is being accepted less readily [7–17]. Attracting less attention is the observation that components of PBMC isolation, especially platelets and monocytes, appear to produce factors which enhance fibroblast and endothelial proliferation [18,19,27,28]. Whether the factors which appear to have opposite effects are produced by the same cell undergoing similar types of stimulatory responses is unknown (for review, see Unanue [29], and Waksman [22]).

The mechanism whereby such factors effect cell proliferation/differentiation is also unknown. Recent studies suggest lymphokines can enhance and suppress fibroblast proliferation, and may correlate with the cell line tested, transformed vs. nontransformed [30,31]. In both studies, lymphokine stimulated collagen production. Lymphokines augment the release of lymphokine, and inhibit proliferation. These lymphokines appear to move cells from the G-O phase to the G-1 phase of the cell cycle. If receptors exist, other lymphokines appear to act in the post-G-1 component of the cell cycle [28,32]. These observations appear to support the rather simplistic concept of the "net effect" being the important *in vivo* correlation. If the "net effect" of mediator release is enhancement of proliferation, this occurs while the opposite occurs if the "net effect" is suppression of proliferation.

Although apparently genetically acquired, psoriasis comes on relatively late, between the ages of 10 and 30 [4]. It flares and remits spontaneously and can be readily induced with specific trauma at times, and not at others [1,4], suggesting modifying or compensatory influences are playing a role in this disease. Unfortunately, there is no method to recognize early events of flare or remission of psoriasis. Thus, the state or potential compensatory mechanisms are unknown in our subjects. There is no correlation between either the inhibition or enhancement of cell proliferation by these supernatants and the delayed intradermal skin tests. Further, there is no correlation between the degree or state of psoriasis, and the abnormal mononuclear cell function (depressed responsiveness to skin tests, mitogen and mixed lymphocyte reactions, or with monocyte chemotaxis or monocyte reduction of NBT) that we and others have previously reported [20,21,33–37].

Supernatants from antigen-stimulated PBMC were utilized, rather than mitogen-stimulated PBMC, because antigens are more physiologic agents. Nonspecific mitogens, such as PHA, can stimulate proliferative events by themselves [31]. Data, not presented, demonstrate that when the antigens used in these experiments were added at various doses (up to 2.5 ×) to the HeLa cell cultures, no effect on 3H thymidine incorporation was noted. There was no correlation between PIF activity and the type of antigen used, an observation supported by similar data seen when another lymphokine (lymphocyte-derived chemotactic factor) was similarly generated and quantitated [21]. Supernatants were tested without further purification to determine "net effect", i.e., whether or not the whole supernatant from PBMC had an overriding influence of one type or another. Distribution of the number of antigens used, i.e., the number of subjects stimulated with SKSD, PPD, etc., amongst the test population, did not account for the reported differences, normal vs. psoriasis. Objections can be raised to our choice of utilizing different antigens; however, an equally, or more, objectionable choice would be the use of the same antigens with their varying responses. In some subjects, some antigens elicited no response; thus, the only choice seemed to be that which we made.

Activity was enhanced by dialysis, suggesting the factor(s) released by antigen-stimulated PBMC effecting cell proliferation has a molecular weight of greater than 10,000 (i.e. they are apparently not prostaglandins or nucleosides). Conditioned media does alter activity; both the inhibitory and proliferative events are more pronounced following dialysis. The exception is undiluted, active supernatants from normals. Data, not presented, from supernatants concentrated times 5, demonstrate only 20% more inhibitory activity than seen with unconcentrated samples. The failure to detect a difference in protein concentration, predialysis compared with postdialysis, suggests that the increase in protein concentration, secondary to dialysis, had a negligible effect on the generation of the data. The nonlinearity of the 5× concentration weighs in favor of several factors being involved in some type of competitive role in our observations.

Most data are expressed as a percent of media, rather than as an index of active vs. control. This was done to facilitate comparison of the response of PBMC of normal and psoriatic subjects to antigenic stimulation. Using this method of comparison, normal vs. psoriasis, another concept emerges; unstimulated PBMC produce factors which effect cell proliferation.

The data of Table II demonstrate that with dilution, factors effecting proliferation are more in evidence. If equal amounts and both had equal effects on cells, values should not exceed 100%. The factor(s) which causes proliferation appears to have a greater effect on HeLa cells than does the factor(s) which causes inhibition (dilutional data and wash experiments—Table V). The effect of supernatants on cell counts (Table IV) supports the use of 3H thymidine as an indicator of HeLa cell proliferation in this assay system.

The failure to see an effect when supernatants were added at 24 hr, a time when confluence of growth is being approached, suggests other factors are emerging as a function of time, which also play a role in the kinetics of cell proliferation in this *in vitro* system. That mononuclear cell supernatants will only influence cell growth during the expotential phase of growth, i.e. 0–20 hours, is suggested by our data. However, definitive data will have to await results of further experiments.

The possibility that the limited number of experiments to quantitate PIF in individuals is not representative appears unlikely. Another lymphokine (lymphocyte-derived chemotactic factor) previously reported [21] was quantitated in these and other psoriatic subjects. A good correlation exists between

the quantitative assessment of these two lymphokines in individual subjects ($r = 0.56$, $p < 0.01$).

The observations reported in this paper support the concept that inflammatory events play a role in the enhancement and suppression of cell proliferation. Whether these events are regulated by separate molecular substances is unknown. We feel the dilutional data (Table II) and the pulse wash experiments (Table V) support separate factors for inhibition and enhancement of HeLa cell proliferation *in vitro*. Using cells from peripheral blood, our data support the possibility that psoriasis may result from an aberration of mediator release; however, more experimental data are needed to permit these conclusions.

REFERENCES

1. Farber EM, RJ Roth, E Aschheim, DD Eddy, WW Epinette: Role of trauma in isomorphic response in psoriasis. Arch Dermatol 91: 246–251, 1965
2. Braun-Falco O: The initial psoriatic lesions, Psoriasis: Proceedings of the Second International Symposium. Edited by EM Farber, EJ Cox. Yorke Medical Books, New York, 1977, pp 1–11
3. Weinstein GD, JL McCullough: Cytokinetics in diseases of epidermal hyperplasia. Ann Rev Med 24:345–352, 1973
4. Farber EM, Nall ML: The natural history of psoriasis in 5,600 patients. Dermatologica 148:1–18, 1974
5. Granger GA, Kolb WP: Lymphocyte in vitro cytotoxicity: Mechanisms of immune and non-immune small lymphocyte mediated target L cell destruction. J Immunol 101:111–120, 1968
6. Lee SC, Lucas ZJ: Regulatory factors produced by lymphocytes. I. The occurrence of multiple α-lymphotoxins associated with ribonuclease activity. J Immunol 117:283–291, 1976
7. Green JA, Cooperband SR, Rutstein JA, Kibrick S: Inhibition of target cell proliferation by supernatants from cultures of human peripheral lymphocytes. J Immunol 105:48–54, 1970
8. Badger AM, Cooperband SR, Green JA: Direct observations on the effect of "proliferation inhibitory factor" on the clonal growth of target cells. J Immunol 107:1259–1267, 1971
9. Holzman RS, Lebowitz AS, Valentine FT, Lawrence HS: Preparation and properties of cloning inhibitory factor. I. Inhibition of HeLa cell cloning by stimulated lymphocytes and their culture supernatants. Cell Immunol 8:249–258, 1973
10. Holzman RS, Valentine FT, Lawrence HS: Preparation and properties of cloning inhibitory factor. II. Factors affecting its production and assay. Cell Immunol 8:259–269, 1973
11. Stewart WE II, Gresser I, Tovey MG, Bandu M-T, Goff SL: Identification of the cell multiplication inhibitory factors in interferon preparations as interferons. Nature 262:300–302, 1976
12. Weinstein Y, Brodeur BR, Melmon KL, Merigan TC: Interferon inhibition of lymphocyte mitogenesis. Immunology 33:313–318, 1977
13. Knight E Jr: Antiviral and cell growth inhibitory activities reside in the same glycoprotein of human fibroblast interferon. Nature 262:302–303, 1976
14. Smith RT, Bausher JAC, Adler WH: Studies of an inhibitor of DNA synthesis and a nonspecific mitogen elaborated by human lymphoblasts. Am J Pathol 60:495–504, 1970
15. Houck JC, Irausquin H, Leikin S: Lymphocyte DNA synthesis inhibition. Science 173:1139–1141, 1971
16. Namba Y, Waksman BH: Regulatory substances produced by lymphocytes. III. Evidence that lymphotoxin and proliferation inhibitory factor are identical and different from the inhibitor of DNA synthesis. J Immunol 116:1140–1144, 1976
17. Lee SC, Lucas ZJ: Regulatory factors produced by lymphocytes. II. Inhibition of cellular DNA synthesis associated with a factor inhibiting DNA polymerase α activity. J Immunol 118:88–97, 1977
18. Leibovich SJ, Ross R: A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. Am J Pathol 84: 501–513, 1976
19. Polverini PJ, Cotran RS, Gimbrone MA Jr, Unanue ER: Activated macrophages induce vascular proliferation. Nature 269:804–806, 1977
20. Krueger GG, Hill HR, Jederberg WW: Inflammatory and immune cell function in psoriasis—A subtle disorder. I. In vivo and in vitro survey. J Invest Dermatol 71:189–194, 1978
21. Krueger GG, Jederberg WW, Ogden BA, Reese DL: Inflammatory and immune cell function in psoriasis. II. Monocyte function, lymphokine production. J Invest Dermatol 71:195–201, 1978
22. Waksman BH, Wagshal AB: Lymphocytic functions acted on by immunoregulatory cytokines. Significance of the cell cycle. Cell Immunol 36:180–196, 1978
23. Boyum A: Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 21:77–89, 1968
24. Lever WR, Schaumburg-Lever G: Pseudocarcinomatous hyperplasia, *Histopathology of the Skin. J. B. Lippincott Co., 5th edition*, Philadelphia, pp 481–483, 1975
25. Marks R: Is there a relationship between clinical morphology and epidermal cell kinetics? Proc R Soc Med 68:3–4, 1975
26. Kurland JI, Kincade PW, Moore MAS: Regulation of B-lymphocyte clonal proliferation by stimulatory and inhibitory macrophage-derived factors. J Exp Med 146:1420–1435, 1977
27. Kohler N, Lipton A: Platelets as a source of fibroblast growth-promoting activity. Exp Cell Res 87:297–301, 1974
28. Slayback JRB, Cheung LWY, Geyer RP: Comparative effects of human platelet growth factor on the growth and morphology of human fetal and adult diploid fibroblasts. Exp Cell Res 110:462–466, 1977
29. Unanue ER: Secretory function of mononuclear phagocytes, A review. Am J Pathol 83:397–417, 1976
30. Johnson RL, Ziff M: Lymphokine stimulation of collagen accumulation. J Clin Invest 58:240–252, 1976
31. Wahl SM, Wahl LM, McCarthy JB: Lymphocyte-mediated activation of fibroblast proliferation and collagen production. J Immunol 121:942–946, 1978
32. Wagshal AB, Waksman BH: Regulatory substances produced by lymphocytes. VII. Cell cycle specificity of inhibitor of DNA synthesis (IDS) action in lymphocytes. J Immunol 121:966–972, 1978
33. Glinski W, Obalek S, Langmer A, Jablonska S, Haftek M: Defective function of T-lymphocytes in psoriasis. J Invest Dermatol 70: 105–110, 1978
34. Guilhou JJ, Clot J, Meynadier J, Lapinski H: Immunological aspects of psoriasis. I. Immunoglobulins and anti-IgG factors. Br J Dermatol 94:501–507, 1976
35. Lischka G: T-lymphocytes and psoriasis. Arch Dermatol Res 257: 107–108, 1976
36. Guilhou JJ, Maynadier J, Clot J, Charmasson E, Dardenne M, Brochier J: Immunologic aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes. Br J Dermatol 95:295–301,
37. Levantine A, Brostoff J: Immunological responses of patients with psoriasis and the effect of treatment with methotrexate. Br J Dermatol 93:659–666, 1975